# Avian Egg: A Multifaceted Biomaterial for Tissue Engineering

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**Abstract**

Avian eggs offer a natural and environmentally friendly source of raw materials, and many of their components hold great potential in tissue engineering. An avian egg consists of several beneficial elements: the protective eggshell, the eggshell membrane, the egg white (albumen), and the egg yolk (vitellus). The eggshell is mostly composed of calcium carbonate and has intrinsic biological properties that stimulate bone repair. It is a suitable precursor for the synthesis of hydroxyapatite and calcium phosphate, which are particularly relevant to bone tissue engineering. The eggshell membrane is a thin protein-based layer with a fibrous structure composed of several valuable biopolymers, such as collagen and hyaluronic acid, also found in the human extracellular matrix. As a result, the eggshell membrane has found several applications in skin tissue repair and regeneration. The egg white is a protein-rich material that is under investigation for the design of functional protein-based hydrogel scaffolds. The egg yolk, composed mainly of lipids, also contains diverse essential nutrients (e.g., proteins, minerals, and vitamins) and has potential applications in wound healing and bone tissue engineering. This review summarizes the advantages and status of egg components in tissue engineering and regenerative medicine as well as their current limitations and future perspectives.

# 1. Introduction

Tissue loss and tissue defects result in approximately eight million surgical interventions each year and represent nearly half of the medical costs in the United States.[1, 2] Traditionally, tissue failures and defects are mainly treated with prostheses, transplantation of donor organs, or the implantation of mechanical devices. However, the lack of adequate and proper donor organs and the inability of prostheses and mechanical devices to restore a patient's normal life highlight the need for alternatives.[3-6] Tissue engineering (TE) has emerged as an attractive alternative for treating tissue malfunctions.[7-9] TE is at the interface of materials science, chemistry, bioengineering, and cell biology. Using principles from these fields, TE aims to develop biological substitutes to maintain, restore, or improve tissue function by combining a scaffold, cells, and biomolecules.[1, 10, 11] Biomaterials play a vital role in TE because they can provide cells with a microenvironment mimicking their native physiological niche, thereby enabling tissue regeneration.[12-14] Biomaterials can be created from synthetic or naturally occurring sources, such as seashells or eggs. Among various sources of materials, those that are naturally derived can recapitulate extracellular matrix (ECM) elements such as cell binding sites, which can ultimately influence the cellular behaviors (e.g., spreading, migration, differentiation) needed for tissue growth both *in vitro* and *in vivo*.[15-17]

Among the various biomaterials used for scaffold design, those with high bioactivity, sustainability, easy handling/processability, and low production costs are preferred.[18-20] The components of avian eggs fulfill these requirements, prompting the interest of many researchers to use them as a source of raw materials.[21-24] Egg constituents are beneficial in the management of various tissue injuries.[25-27] In fact, their use in wound healing and burn treatment can be found in manuscripts from ancient Persian, Chinese, Egyptian, and Roman civilizations.[28-30] In addition to their advantageous intrinsic bioactive features, sustainability, ease of handling, and affordability, avian egg elements are naturally degradable.[28-30]

The avian egg has four main components: (*i*) a protective eggshell (ES) composed mainly of calcium and phosphate (CP), (*ii*) a thin ES membrane (ESM), which has a fibrous structure and contains proteins similar to those found in human ECM, (*iii*) an egg white (EW), a major source of proteins and bioactive peptides, and (*iv*) an egg yolk (EY), which is composed mostly of lipids but includes essential elements such as proteins, polysaccharides, minerals, and vitamins (Figure 1).[31-33] In this review, we describe the utility and advantages of each egg component for TE and regenerative medicine, its current limitations, and future directions for clinical translation.

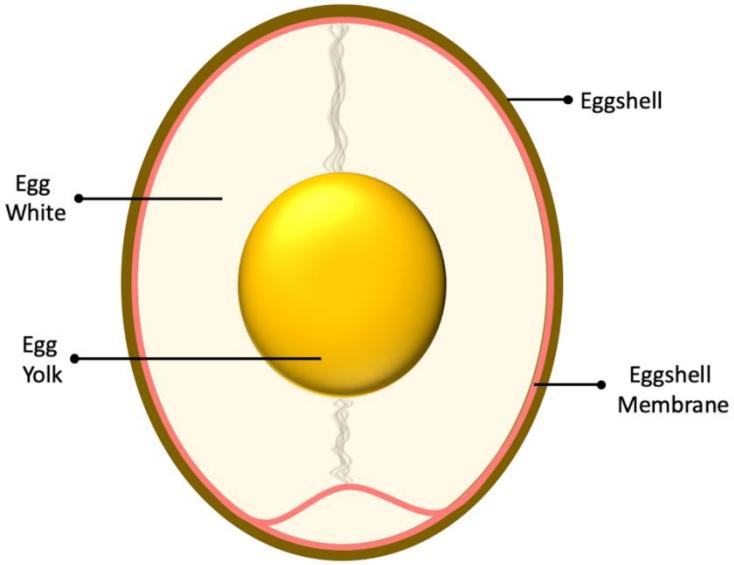


**Figure 1. The structure of a chicken egg.** Major components of a chicken egg include a calcified ES, a thin ESM, a viscous protein-rich EW, and a lipid-rich EY bulge.

## 2. Eggshell

### Properties of ES and its similarities with human bone

Chicken egg farmers, chick hatcheries, egg-breaking plants, food-processing industries, and household consumption produce millions of tons of ES every year.[34-36] Disposal of ES waste can be costly and may pose some environmental concerns.[8] As a biomaterial waste, ES is cheap

and abundantly available, making it a suitable for several purposes. Moreover, recycling this naturally derived biomaterial into other industries could offer an affordable and sustainable source.[37, 38] Applications of avian ES waste include removal of heavy metals from industrial wastewater,[39, 40] fabrication of products such as biocatalysts,[41, 42] Li-ion batteries,[43] fertilizers,[44] ceramics,[45, 46] thermal insulators,[47] and composite fillers,[48] as well as biomedical applications such as TE.[49] ES is a natural composite material that represents about 10% of the total egg weight.[50] It is composed of 94% calcium carbonate ($CaCO_3$), 4% organic matter, 1% calcium phosphate [$Ca_3(PO_4)_2$], 1% magnesium carbonate ($MgCO_3$), and trace amounts of sodium (Na, 1512 ppm), magnesium (Mg, 3472–4500 ppm), strontium (Sr, 320–411 ppm), potassium (K, 524 ppm), and sulfur (S, 589 ppm).[51-54]

Natural human bone consists of two types of calcium phosphate: calcium-deficient hydroxyapatite (HAp) and calcium-deficient β-tricalcium phosphate (βTCP).[55-59] The chemical structure of pure HAp is $Ca_5(PO_4)_3(OH)$ with a calcium to phosphate ratio of 1.67, while pure βTCP is β-$Ca_3(PO_4)_2$ with a calcium to phosphate ratio of 1.5. In bone, these molecules contain trace elements, including Na, Mg, Sr, K, and Si (silica).[45, 60] Thus, compared to HAp or βTCP synthesized from pure reagents, these compounds when isolated from natural sources like animal bone, coral, seashell, snail shell, pearl, and ES, are non-stoichiometric and calcium-deficient due to the presence of trace elements. This non-stoichiometry is advantageous for bone tissue scaffolds.[61, 62] The trace elements found in ES are also present in bone tissues and are known to promote angiogenesis and osteogenesis.[60] As a result, ES and ES-derived calcium phosphate, HAp, or βTCP have been used to design bone TE scaffolds.[63-65]

## ES as a source of calcium phosphate

Dadhich *et al*. developed three-dimensional (3D)-printed scaffolds using the naturally occurring polymer chitosan and calcium phosphate as the printing material.[66] One source of calcium phosphate they investigated was derived from ES. They compared the characteristics of the fabricated scaffolds with ES-derived calcium phosphate (ES-CaP) or a chemically

synthesized calcium phosphate (CS-CaP). To evaluate their biocompatibility, these scaffolds were seeded with primary human mesenchymal stem cells (hMSCs). Compared to CS-CaP scaffolds, cellularized ES-CaP scaffolds exhibited enhanced proliferation, filopodial growth, metabolic activity, and differentiation toward an osteoblast lineage. Based on the promising *in vitro* data with cellularized ES-CaP scaffolds, the authors subcutaneously implanted these biomaterials into rabbits. The ES-CaP scaffolds were biocompatible and provided a suitable environment for tissue growth and vascularization.

In a second study, Huang *et al*. supplemented ES powder with magnesium oxide (MgO).[67] MgO was added to provide $Mg^{2+}$, an important ion that controls a myriad of cellular processes, including the functional properties of integrins which play a major role in anchoring cells to the ECM. Specifically, $Mg^{2+}$ promotes osteoblast proliferation, matrix mineralization, and vascularization within the scaffolds.[68, 69] To fabricate a bone TE scaffold, ES powder was combined with MgO to form a nanocomposite material (ES/MgO), which was subsequently mixed with carboxymethyl chitosan (CMC) with or without bone morphogenetic protein 2 (BMP2). BMP2 is a natural signal that promotes osteogenesis and ultimately bone formation.[70, 71] To obtain a stable scaffold, the composite (ES/MgO/CMC +/- BMP2) was crosslinked through carbodiimide chemistry (Figure 2a). The addition of ES/MgO to CMC significantly improved the Young's modulus and compressive strength of the resulting scaffold (Figure 2b). Next, the scaffolds were seeded with human adipose-derived mesenchymal stem cells (hADSCs). When implanted in mice, these cellularized scaffolds released BMP2 and $Mg^{2+}$ over 2 and 4 weeks, respectively, stimulating various signaling pathways associated with osteogenesis. Notably, both ES-derived $CaCO_3$/MgO/CMC and ES-derived $CaCO_3$/MgO/CMC/BMP2 scaffolds displayed improved bone regeneration when compared to CMC scaffolds (Figure 2c). However, the specific role of ES-derived $CaCO_3$ on bone tissue regeneration was not investigated in this study.

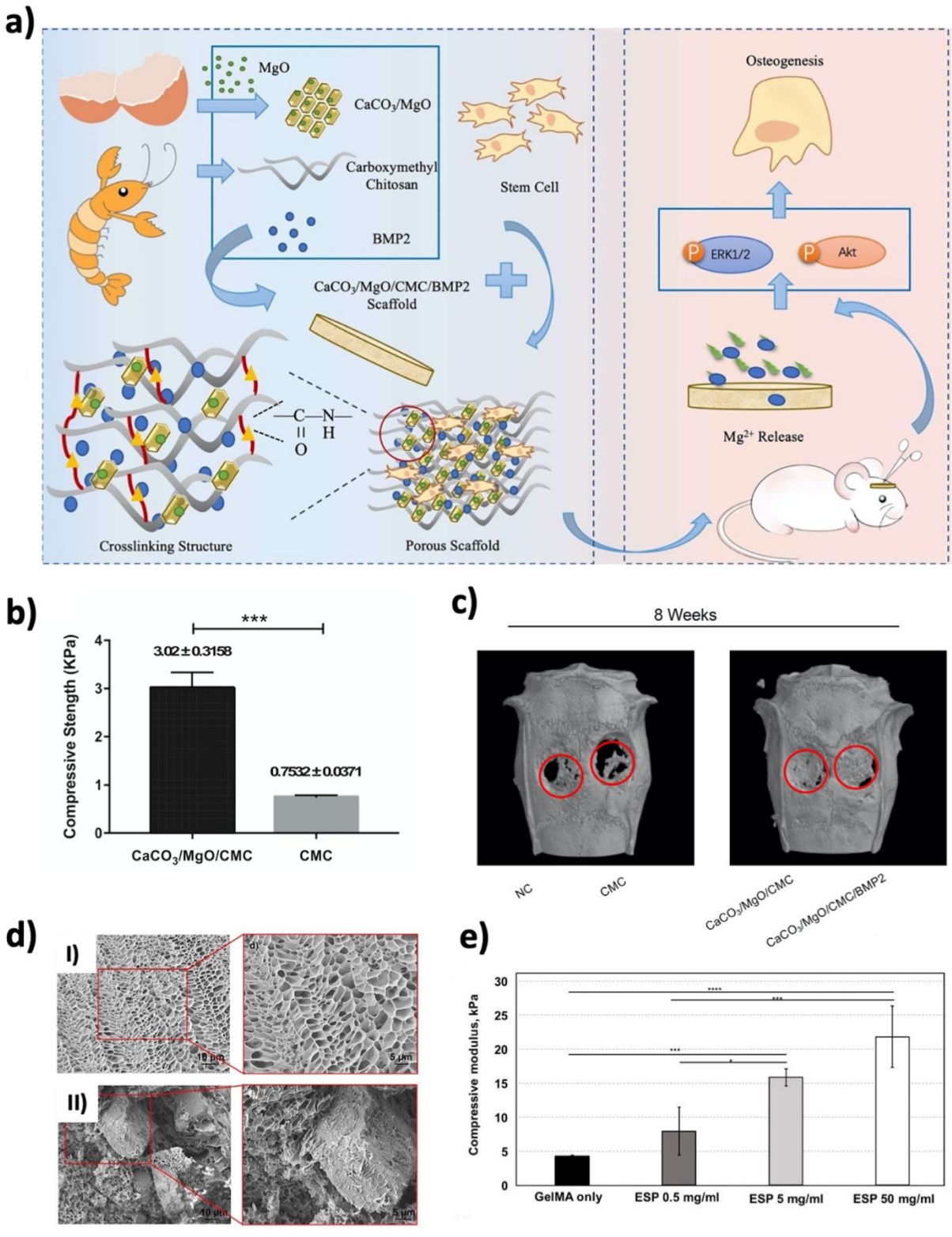


**Figure 2. The intrinsic similarity of ES to the inorganic component of bones makes it a good candidate material for bone TE.** a) Schematic depicting the preparation and characterization of ES-derived $CaCO_3$/MgO/CMC/BMP2 ($CaCO_3$/MgO/CMC/BMP2)

scaffolds. b) Comparison of the mechanical strength of ES-derived $CaCO_3$/MgO/CMC ($CaCO_3$/MgO/CMC) and CMC scaffolds. c) High-resolution micro-CT imaging of scaffold-mediated bone repair in critical-sized rat calvarial defects 8 weeks after implantation: NC (negative control), CMC, $CaCO_3$/MgO/CMC, and $CaCO_3$/MgO/CMC/BMP2. (a–c) Reproduced with permission from reference 67. Copyright 2020 Elsevier BV. d) Scanning electron microscopy images of (I) pure GelMA hydrogel and (II) GelMA hydrogels reinforced with ES particles (GelMA-ESP). e) Comparison of the compressive modulus of GelMA and GelMA-ESP hydrogels at different ESP concentrations. Reproduced with permission from reference 72. Copyright 2019 The Royal Society of Chemistry.

In a separate study, Wu *et al*. combined ES with gelatin methacrylate (GelMA) to form GelMA-reinforced ES particle (GelMA-ESP) hydrogels as potential bone TE scaffolds (Figure 2d).[72] ES was added to the hydrogels to reinforce the mechanical stiffness of the scaffolds, which would ultimately enhance the adhesion, proliferation, growth, and osteogenic differentiation of pre-osteogenic cells. The compressive moduli of the resulting hydrogels increased proportionally to the amount of incorporated ES (Figure 2e). The *in vitro* studies showed that, compared to GelMA hydrogels, GelMA-ESP scaffolds promoted not only osteogenic differentiation but also enhanced cell proliferation and mineralization. *In vivo*, the subcutaneous implantation of cell-free hydrogels for 14 days showed that GelMA-ESP hydrogels had improved biocompatibility and increased biodegradation compared to GelMA hydrogels.

## ES as a source of HAp

HAp is a suitable material for bone TE since it can reinforce the mechanical strength of scaffolds and exhibits excellent osteoconductivity (i.e., promotes bone growth) and osteoinductivity (i.e., induces osteogenic differentiation). Not only is HAp bioactive, but it is also biodegradable, biocompatible, and non-immunogenic.[64, 73, 74] It can be synthesized from

synthetic or natural materials. The use of synthetic materials for HAp synthesis is expensive and results in stoichiometric HAp.[75, 76] Such stoichiometric HAp differs from the calcium-deficient HAp found in human bone.[77] Thus, natural and readily available sources for the synthesis of calcium-deficient HAp, such as ES, are desirable. [78, 79]

HAp can be produced from ES by various methods, including but not limited to the sol-gel process, solid-state reaction, aqueous precipitation, hydrothermal technique, and microwave irradiation. To prove that HAp could be sustainably synthesized from biowastes (ES and urine), Ronnan *et al*. used an electrochemical technique to produce HAp from waste ES and synthetic urine.[80] The synthesis method substantially influences the morphological properties—shape, size, and crystallinity—of the resulting HAp.[81-83] Hydrothermal synthesis is a direct and widely used method for the synthesis of HAp from ES. However, it is a time-consuming and arduous technique and does not provide good control over HAp morphology.[84] Microwave irradiation is an effective method of producing HAp from ES. Compared with the hydrothermal technique, microwave irradiation has several advantages, such as scalability, exquisite control over HAp morphology, and faster production turnaround.[76, 79] HAp particle size is an important feature and several approaches have been developed to control their diameters when synthesized from ES.[79, 85] Unlike microsized HAp, nanosized HAp exhibits higher protein adsorption and enhanced cellular responses such as osteoblast adhesion and proliferation.[82, 84] The biological properties of nanosized HAp may be due to their resemblance to the natural HAp typically deposited during bone mineralization.[38] Kattimani *et al.* used ES-derived nanosized HAp in a randomized controlled clinical study for bone regeneration using a socket-defect model.[86] Nanosized HAp was made using the microwave irradiation technique. They monitored bone regeneration in twelve patients over six months. Compared to the control group, bone regeneration was significantly better in groups with ES-derived nanosized HAp grafts at early stages and after complete healing. Altogether, the results indicate that ES-derived nanosized HAp is affordable, safe, and possesses excellent bone-healing properties.

Although HAp offers several advantageous features for bone TE, it has several limitations such as slow degradation, low fracture toughness, and high brittleness. These properties have restricted the use of solid HAp in scaffold design, especially for load-bearing bone defects.[78, 87] To overcome these limitations, HAp ceramic-based and HAp polymer-based composite scaffolds have been investigated.[88-90] Carbon nanotubes and silica have been used to produce HAp ceramic composite scaffolds.[91-93] Natural polymers, such as gelatin, starch, collagen, bacterial cellulose, alginate, and chitosan, as well as the synthetic polymers polycaprolactone (PCL), polylactic acid (PLA), and polyvinyl alcohol (PVA), have been used to fabricate HAp polymer composite scaffolds.[89, 94, 95]

In two studies, Trakoolwannachai *et al*. described the application of ES-derived HAp for bone tissue engineering. In this approach, ES-derived HAp was first synthesized by the wet chemical precipitation method and its biocompatibility was tested with Saos-2 cells, a human osteoblast-like cell line. The synthesized ES-derived HAp was then combined with a synthetic polymer (PCL) in one study,[96] and with chitosan, a naturally derived polymer, in the other study.[97] PCL is an intrinsically hydrophobic polymer that has a low capacity to absorb water and swell and is degraded slowly *in vivo* (up to 3 years).[98, 99] The addition of ES-derived HAp into the PCL matrix slightly increased the swelling and degradation properties of the resulting scaffolds by allowing water to permeate the interface between HAp and PCL. Seeding the HAp-PCL composite scaffolds with Saos-2 cells showed that cells attached to the constructs successfully and remained viable. Unlike PCL, chitosan is a hydrophilic, naturally derived polymer.[97] The addition of ES-derived HAp into chitosan scaffolds decreased the swelling ratio and increased the surface roughness. Compared with tissue culture plastic, chitosan and HAp-containing chitosan scaffolds led to a decreased cell-matrix interaction, although the Saos-2 cells proliferated on both scaffold types. For a potential clinical translation, the performance of these ES-derived HAp scaffolds in comparison to pure PCL- or chitosan-based scaffolds needs to be investigated in relevant preclinical models.

## ES as a source of TCP

βTCP is another calcium phosphate suitable for TE because of its advantageous mechanical and biodegradable properties. [57, 100, 101] Unlike HAp, βTCP has a faster biodegradation rate and, if released into tissue fluids, is resorbed by cells. These properties make βTCP a great candidate in bone TE because it can be replaced by the newly formed bone tissue.[102-104] Like HAp, βTCP is osteoconductive and osteoinductive,[105] and various methods are available for βTCP synthesis from ES, such as wet precipitation, solid-state synthesis, and thermal conversion.[100, 101, 103]

For instance, Roopavath *et al.* fabricated ceramic scaffolds from nanosized phase-stable βTCP synthesized from avian ES, and evaluated their applicability in tissue engineering.[101] For βTCP synthesis, a wet chemical precipitation method was applied with or without ball milling. The results showed that pre-grinding of ES for 15 h reduces the required temperature for chemical reaction. For ceramic scaffold preparation, synthesized βTCP was mixed with a 5% PVA solution, then polyurethane foams were immersed in the solution and sintered at 1000 ºC to remove polymers and form the ceramic scaffolds. The *in vitro* cell viability assay indicated that βTCP ceramic scaffolds have good biocompatibility and great potential for use in tissue engineering.

Composite scaffolds made from ES-derived βTCP and synthetic polymers, such as PCL and PLA, have also been investigated. For example, Shafiei *et al*. incorporated carbon dots, ES-derived βTCP, or both, into nanocomposite scaffolds of PCL and PLA.[106] For scaffold preparation, they electrospun a PCL/PLA solution containing βTCP and/or carbon dots. In a cell-free degradation assay, the addition of βTCP slightly increased scaffold degradation. Because βTCP can also be degraded by cells, comparing the degradation in the presence of cells or after implantation could have revealed a greater effect of βTCP inclusion on scaffold stability. *In vitro* investigations with human buccal fat pad-derived stem cells showed that carbon dot-containing ES-derived βTCP scaffold led to the highest cell proliferation rate and osteogenic activity.

# 3. Eggshell membrane

## Extraction and Properties of ESM

The ESM is a thin, protein-based layer between the ES and the EW. Similar to the human amniotic membrane, this layer protects the growing embryo, has antibacterial properties, and provides a structural foundation for the calcification of the ES during the different stages of embryonic development.[29, 107] Like ES, ESM is considered an industrial waste product and is available in large quantities at low cost.[108, 109] The inner part of the ESM is readily separated from the rest of the egg, but the outer portion adheres to the ES and thus is more difficult to separate. On an industrial scale, the efficiency of this process is important, and various strategies such as mechanical separation combined with mild acid treatment can effectively peel the ESM from the ES.[109-111]

ESM is mostly composed of collagenized fibrous proteins (Figure 3). This unique fibrous protein network is biocompatible, interwoven, and porous, exhibiting high water permeability.[112-114] For centuries, ESM has been used for treating chronic ulcers and bone fractures, and was formally named "phoenix cloth" in Chinese traditional medicine.[28, 110, 115, 116]

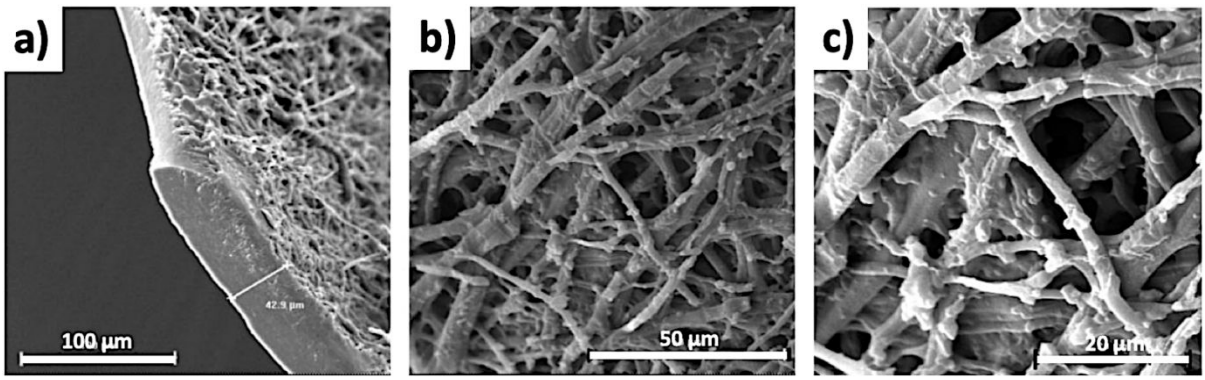


**Figure 3. Microstructure of ESM.** Scanning electron micrograph of an ESM showing (a) side view and (b, c) top view. Reproduced with permission from reference 117. Copyright 2008 John Wiley & Sons, Ltd.

ESM is mostly composed of proteins (~90%), including collagen type I, collagen type V, collagen type X, glucosamine, desmosine, and keratin. Lipids comprise ~3% and carbohydrates ~2%, with the most abundant being glycosaminoglycan hyaluronic acid.[111, 118-122] The similarity of ESM to human ECM, as well as the presence of various functional groups like amines, amides, and carboxylic acids, makes it a promising material for TE, biosensors, and drug delivery.[122-126] Other applications of ESM have been reported, including enzyme immobilization, removal of heavy metals, and as components of fuel cell membranes and dietary supplements.[127-129]

## Applications of ESM in TE

ESM has been used to create scaffolds with potential application in nerve regeneration, skin TE, vascular TE, and bone TE. In each case, ESM combined with other molecules or polymers was superior to ESM alone. To generate a scaffold with properties compatible with peripheral nerve repair, Golafshan *et al*. developed a double network composite using polycaprolactone fumarate (PCLF) and manually extracted ESM.[130] They used a vacuum infiltration approach to create double network scaffolds by soaking ESM films in PCLF solutions containing either dichloromethane or acetic acid. Acetic acid achieved a greater incorporation of PCLF into ESM films (Figure 4a) and was associated with the lowest swelling rate and highest tensile strength. To evaluate their biocompatibility, pheochromocytoma 12 (PC12) cells, a classical neuronal cell model, were seeded on ESM-PCLF scaffolds. While ESM-PCLF scaffolds prepared in acetic acid exhibited improved cell attachment, cells proliferated similarly on both ESM-PCLF and ESM scaffolds. Furthermore, the PC12 cells grown on ESM-PCLF scaffolds spread rapidly and formed intercellular connections, demonstrating their potential for nerve regeneration.

For skin tissue regeneration, smooth bilayered scaffolds constructed on ESM were superior to plain ESM.[131] Ray *et al*. coated ESM with nanofibers to create smooth bilayered

scaffolds by direct electrospinning of a PCL and chitosan solution onto the surface of ESM that had been extracted with dilute acetic acid.[131] Compared to non-coated ESM, nanofiber-coated ESM showed reduced surface roughness, slower enzyme-mediated degradation, greater hydrophobicity, and was associated with increased tensile strength. Water retention and water vapor transmission capacity of nanofiber-coated ESM were consistent with the profile needed for maintaining wound hydration while retaining a stable shape. *In vitro* investigations with human dermal fibroblasts revealed that the nanofiber coating enhanced cell attachment, spreading, and proliferation at early time points and accelerated cell infiltration into the scaffold at later times (Figure 4b). When compared to non-coated ESM, nanofiber-coated ESM also demonstrated improved antibacterial activity. Next, non-coated and nanofiber-coated ESMs were tested in full thickness skin wounds in rats. Consistent with their biophysical properties, wounds treated with nanofiber-coated ESM remained moist. Furthermore, with this biomaterial, wound healing was faster, with efficient re-epithelization and hair re-growth.

In another study, nanofiber-coated ESM has been developed for vascular TE. Instead of the sheet-like structure used for skin grafts, vascular grafts should be designed with a tubular morphology. Yan *et al.* designed small-diameter artificial vascular grafts using thermoplastic polyurethane (TPU) nanofibers that were electrospun onto ESM tubes and subsequently coated with polydopamine and heparin via layer-by-layer deposition. In this approach, they combined the bioactivity of ESM as the internal membrane and the tunable mechanical properties of TPU as the external layer. Furthermore, this strategy led to the formation of wavy small-diameter ESM/TPU tubular-shaped scaffolds with adhesive and anticoagulant properties (Figure 4c).[132] Notably, the wavy structure of these ESM/TPU double-layered vascular grafts reproduced the mechanical behavior of a natural blood vessel, an important feature for bearing blood pressure when introduced into the body. When seeded with human umbilical vein endothelial cells (HUVECs), the internal membrane supported cell attachment and proliferation, and

importantly, the use of heparin reduced platelet attachment, suggesting antithrombogenic properties. Although promising, these biomaterials still need to be tested in relevant animal models to assess their safety, performance, and ultimately clinical potential. In this manner, Biovotec has developed the DermaRep™ as an affordable wound-care dressing to enhance granulation modulation of excess protease action.[133] They are using Purified Eggshell Membrane Protein, or PEP™ as a replacement of mammalian-derived collagen. Based on their report, a First-in-Human trial of DermaRep™ is ongoing in the UK for the treatment of ulcers.

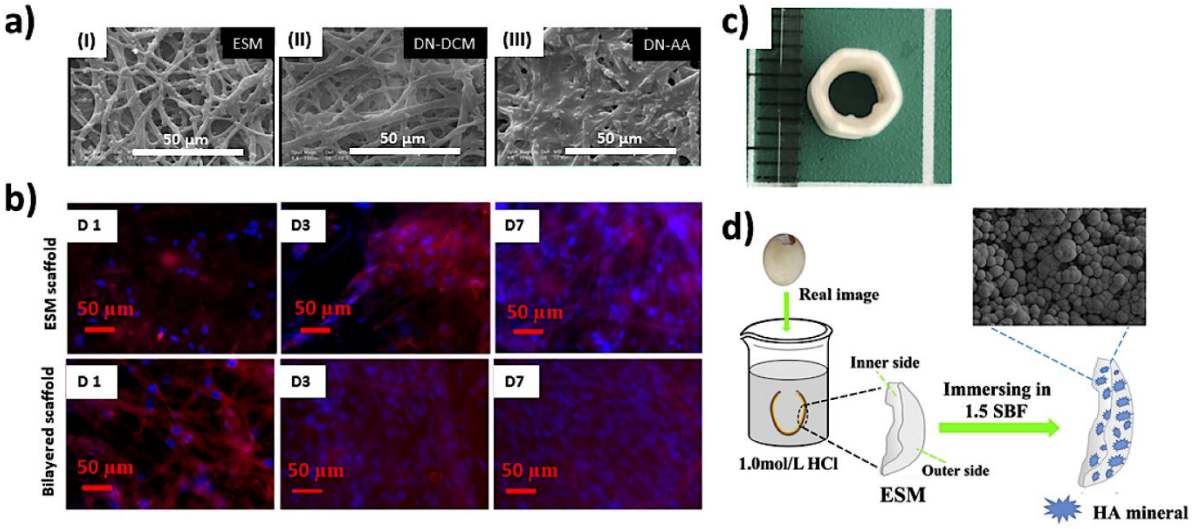


**Figure 4. Leveraging ESM for TE scaffolds.** (a) Scanning electron microscopy images showing the microstructure of (I) ESM and PCLF-coated ESM prepared with PCLF dissolved in (II) dichloromethane or (III) acetic acid. Reproduced with permission from reference 130. Copyright 2017 IOP Publishing Ltd. (b) Fluorescent imaging of human dermal fibroblasts showing their proliferation over 1 week incubation on ESM and nanofiber-coated ESM scaffolds. Reproduced with permission from reference 131. Copyright 2018 American Chemical Society. (c) Photograph showing the cross section of a bilayered ESM/TPU vascular graft. Reproduced with permission from reference 132. Copyright 2020 Elsevier BV. (d) Schematic depicting *in situ* mineralization of HAp on the ESM. Reproduced with permission from reference 134. Copyright 2019 Elsevier BV.

For bone TE, Chen *et al*. used ESM as a promising template for inducing biomimetic mineralization. HAp was successfully generated when the ESM was incubated with a simulated body fluid containing several ions, including $Ca^{2+}$, $Cl^{-}$, $Na^{+}$, $HPO_4^{2-}$, $K^{+}$, $Mg^{2+}$, $SO_4^{2-}$, and $HCO3^{-}$ (Figure 4d).[134] Next, they characterized the properties of their HAp-modified and unmodified ESM and compared both the inner (EW side) and outer (ES side) for supporting MC3T3-E1 cell responses.[134] Unlike unmodified ESM, mineralization occurred on both sides (inner and outer) of the HAp-modified ESM and this platform supported greater cell adhesion and proliferation and promoted osteogenic capacity of MC3T3-E1 cells as well as the expression of bone-related genes and proteins. These findings suggest that ESM-based materials have great potential for bone tissue repair.

## Applications of soluble ESM-derived proteins in TE

ESM contains crosslinked disulfide bonds, limiting its solubility in conventional and non-toxic solvents.[135, 136] Although combining ESM with various polymers has helped, its insolubility makes its processability quite challenging. Even if successful, it is still difficult to control the morphological and mechanical properties of ESM-based scaffolds, impeding their potential use in TE.[137-139] To tackle this problem, cleavage of the disulfide bonds has been used to obtain soluble ESM, mostly composed of soluble ESM protein (SEP). Two main methods of ESM solubilization exist: (*i*) chemical treatments in strong acidic or basic conditions at high temperatures and (*ii*) enzymatic modification, which requires milder conditions.[110, 140] Depending on the solubilization process, water-soluble or water-insoluble SEP is obtained. Water-insoluble SEP is soluble in organic solvents, such as acetic acid and hexafluoro-2-propanol.[110, 140] For instance, Yi *et al*. developed a method to produce water-insoluble SEP.[107] This process used 3-mercaptopropionic acid as a reducing agent in dilute acetic acid, exposure at high temperatures, and neutralization with sodium hydroxide.[107] To improve their mechanical properties, both forms of SEP (water-soluble and water-insoluble) have been blended with synthetic polymers such as PLA, PVA, PCL, poly (lactic-co-glycolic acid), and

poly (ethylene oxide) (PEO) or with naturally derived materials, such as agarose, ES powder, and chitosan.[141-143]

Several studies have used the 3-mercaptopropionic acid-extraction method to develop SEP-containing scaffolds for cartilage, skin, and bone TE.[144-147] Been *et al*. combined SEP with agarose to fabricate a scaffold for cartilage repair and regeneration.[144] Compared to pure agarose scaffolds, agarose-SEP scaffolds had faster degradation. *In vitro* studies with rabbit-derived chondrocytes showed that the incorporation of SEP into the scaffold increased cell proliferation and survival, resulting in higher chondrogenic gene expression and increased synthesis and deposition of ECM proteins. Amirsadeghi at *al.* fabricated a multilayer electrospun scaffold containing SEP mixed with PVA/chitosan in the outer layers and zinc oxide nanoparticles in the inner nanofiber layer. Next, they tested these 3-layered scaffolds with human dermal fibroblasts for skin TE.[145] *In vitro* studies showed that these 3D scaffolds promoted greater cell proliferation than cells cultured on 2D tissue culture plastic. This set of data suggests that SEP has great potential for the design of TE scaffolds. In two other studies, Mohammadzadeh *et al.* used electrospinning to fabricate PCL/silk fibroin-based scaffolds containing SEP for skin TE.[138, 146] They showed that SEP improves nanofiber wettability and degradation rate. They also indicated that these SEP-containing nanofibrous scaffolds are biocompatible and good candidates for skin TE. Kim *et al.* investigated the effect of SEP coating on PCL patches for bone TE. SEP-coating improves PCL patches' wettability and mechanical properties.[147] Moreover, osteoblast culture on scaffolds revealed that SEP-coating improves cell proliferation, biomineralization, and morphology. The *in vivo* mouse calvarial bone defect study has also shown that SEP-coating promotes bone regeneration.

# 4. Egg white

## Properties of EW and EW-derived proteins

EW, also known as albumen, has multiple applications. It represents approximately 56% of the protein in the egg.[148, 149] In the food industry, it is used as a raw material for the preparation of other products and is particularly useful as stabilizer. In medicine, historically, the antibacterial, anti-inflammatory, and healing properties of EW make it useful for wound healing.[150, 151] In addition, EW is cheap, abundant, and easily processed.[152] Moreover, it is nontoxic, biodegradable, and bioactive. These properties suggest that EW is a biomaterial of choice for TE and regenerative medicine.[153] EW or EW-derived proteins are particularly attractive for the design of protein-based hydrogels, as EW-derived proteins are inherently bioactive and possess features similar to the human ECM, such as good porosity and high water uptake.[154, 155]

EW is a mixture of proteins, carbohydrates, vitamins, and minerals.[30] Among the more than 150 proteins in EW, the most abundant include ovalbumin (44.5 kDa, ~54%), conalbumin (also known as ovotransferrin, 77.7 kDa, ~12%), and ovomucoid (28 kDa, ~11%), globulins (~8%), ovomucin (~3.5%), lysozyme (~3.4%), and flavoprotein (~0.8%) (Figure 5a).[152, 156-158] Ovalbumin, conalbumin, and ovomucoid contribute to EW viscosity, and ovalbumin and conalbumin undergo thermally-induced gelation.[30, 156] Furthermore, ovalbumin leads to the foaming properties of EW.[159-161] Conalbumin has antibacterial properties.[162] Ovomucoid has allergenic properties and is extremely resistant to heat, enzymatic denaturation, and degradation.[163-166] Like conalbumin, lysozyme displays antibacterial properties,[167-169] but unlike conalbumin, it is thermally stable.[170, 171] EW-derived components, individually or in combination, hold great promise in the design of functional biomaterials for TE.

## Applications of EW and EW-derived proteins in TE

Compared with other albumins that are widely used in the biomedical field, including bovine serum albumin (BSA), ovalbumin is more affordable. Like other albumins, ovalbumin binds to various proteins, including growth factors, a quality that is advantageous for TE

applications.[151, 156, 161] Farrar *et al*. used partially pure, commercially available chicken ovalbumin to develop porous hydrogels for bone TE and showed that, using *in vitro* studies with MC3T3-E1 cells, the scaffolds supported cell proliferation and promoted their osteogenic differentiation.[172] Other approaches involve combining ovalbumin with synthetic polymers and naturally derived polysaccharides, such as PVA, polyethylene glycol (PEG), cellulose, and starch.[173, 174]

As a stable enzyme with antimicrobial activity, lysozyme has been incorporated into polymer formulations for skin regeneration and into coatings for prosthetic implants.[175, 176] Lysozyme has also been used to form hydrogels for bone TE. Kim *et al*. chemically modified glycol chitosan and lysozyme to form methacrylated glycol chitosan (MeGC) and methacrylated lysozyme (MeLyz), respectively. Next, these biomolecules were crosslinked to fabricate hydrogels with tunable degradation rates.[177] In contrast to pure MeGC hydrogels, the addition of lysozyme allowed MeGC hydrogels to degrade much faster. *In vitro* studies with bone marrow stromal cells showed that the lysozyme-containing hydrogels supported cell proliferation, and they enhanced osteogenic differentiation and infiltration of cells into constructs (Figure 5b). When tested *in vivo* for cranial bone repair, the lysozyme-containing hydrogels promoted greater bone regeneration than pure MeGC hydrogels, as indicated by larger bone growth area, formation of osteoid development at the wound boundary, and by greater wound closure.[177]

EW also has a potential application in the development of TE scaffolds, in which EW is incorporated with other naturally derived biomaterials, such as silk fibroin, bone-derived HAp, and ES. You *et al*. examined the properties of EW-containing films developed with different ratios of silk fibroin and EW.[178] Higher silk content increased their mechanical strength, whereas a higher EW content enhanced elasticity. *In vitro* studies with HUVECs showed that the presence of EW supported cell adhesion and proliferation on the films (Figure 5c). Owuor *et al*. created a solution composed of bone-derived HAp, EW, and diethylenetriamine.[179] With this solution, they formed composite scaffolds with layered structures similar to those found in mother-of-pearl. Within these layered structures, the

crosslinked EW acted as a glue that provided elastic recovery and low deformability to HAp. The EW/HAp composite scaffolds were exceptionally strong (modulus up to 180 GPa) and exhibited high tensile strength and load-carrying capacity. As a proof-of-concept, using 3D-printed bone and ear molds, they fabricated tissue-like structures with mechanical properties similar to the native biological tissues.

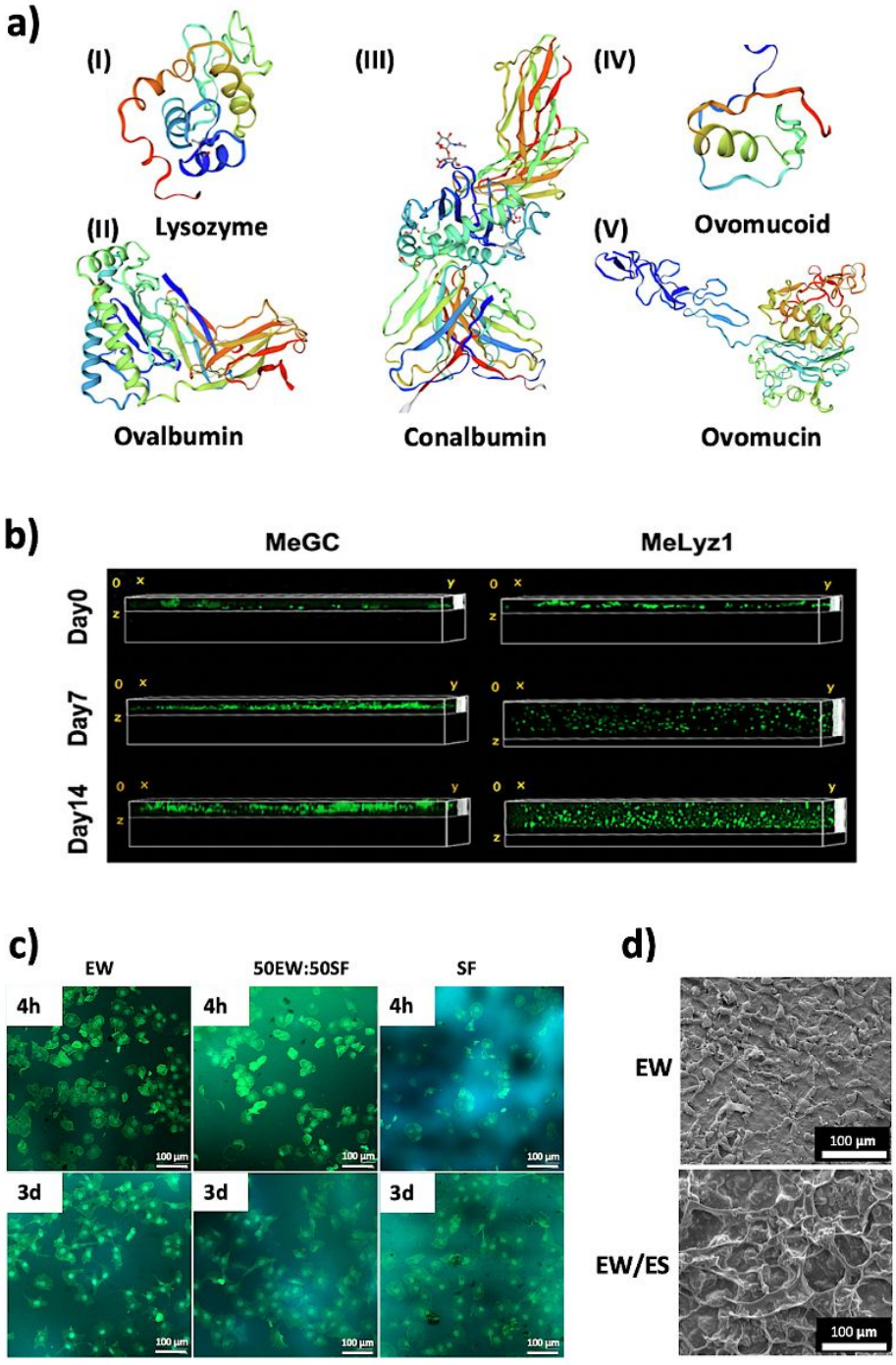


**Figure 5. Application of EW in TE.** (a) Structures of EW-derived proteins. Reproduced with permission from reference 30. Copyright 2020 Elsevier BV. (b) Infiltration of bone marrow

stromal cells into MeGC and lysozyme (MeLyz1)-containing MeGC hydrogels over 14 days. Reproduced with permission from reference 177. Copyright 2018 American Chemical Society. (c) Fluorescent images of HUVECs cultured on different films showing cell spreading on the surface at various time points. Reproduced with permission from reference 178. Copyright 2017 Elsevier BV. (d) SEM images depicting human dental pulp stem cells cultured on EW and ES-loaded EW hydrogels. Reproduced with permission from reference 180. Copyright 2019 American Chemical Society.

ES and EW resemble the inorganic and organic phases of bone, respectively, sparking interest in combining these components for bone TE. Huang *et al*. developed hydrogels using photopolymerizable GelMA in combination with EW or EW/ES.[180] Although EW and EW/ES hydrogels had similar mechanical properties, EW hydrogels exhibited a higher swelling ratio. Both hydrogels supported attachment and proliferation of human dental pulp stem cells (hDPSCs) (Figure 5d), but ES-loaded EW hydrogels promoted osteogenic differentiation to a greater extent. Interestingly, both hydrogels stimulated macrophages, suggesting they could induce an inflammatory response *in vivo*. They also had a fast degradation rate—up to 75% resorption within 10 days. Whether a potential inflammatory response in combination with rapid degradation may promote or impair tissue repair in *vivo* remains to be tested.

# 5. Egg yolk

## Properties of EY

EY consists of water (~50%), lipids (~30%), proteins (~16%), carbohydrates (~4%), and traces of important vitamins (A, B, D, E, K, etc.) and essential minerals (calcium, iron, zinc, magnesium, potassium, sodium, etc.).[181-183] EY can be separated into an aqueous phase (plasma) and an insoluble phase (granules). The plasma phase contains ~85% low-density lipoproteins (LDLs) and ~15% livetin, and the granules contain ~70% high-density lipoproteins (HDLs), ~ 16% phosvitin, and ~ 12% LDLs.[33, 184-186] The combination of these substances, which originally serves as the nutrition source for the embryo, makes it a great

candidate for TE, regenerative medicine, and drug delivery.[187-189] Phosvitin, known to play a role in osteoblast differentiation and mineralization, is particularly relevant for bone TE.

## Applications of EY in TE

Despite the fact that EY has mostly been investigated for drug delivery applications,[190, 191] its application to TE has been reported in limited studies. For example, Charbonneau *et al.* investigated the combination of EY plasma with EW hydrogel for salivary gland TE.[192] They demonstrated that EY plasma can be gelled through a freeze–thaw process. The rheological data proved that the gel's elastic modulus increases over repeated freeze–thaw cycles during 30 days of the experiment. Moreover, they showed that the combination of EY plasma and EW is printable, biocompatible, and is a good candidate for salivary gland and other TE purposes.

Phosvitin is a highly phosphorylated protein, resulting in a strong negative charge that enables the protein to bind to metals and minerals.[193-195] These properties also enable phosvitin to function as a mineral crystal nucleation agent.[196] For instance, phosvitin is known to facilitate the transformation of calcium phosphate into HAp, potentially promoting mineralization of pre-cellularized scaffolds for bone tissue repair. Liang *et al*. fabricated nanofibrous cellulose mats which were subsequently coated layer-by-layer with EY-derived phosvitin and chitosan to create alternating bilayers. Next, these chitosan/phosvitin-coated bilayered mats were tested for mineralization potential.[197] Incubation in a synthetic body fluid resulted in complete coverage of HAp on the surface of the nanofibrous mats, especially for those with the greatest number of layers (Figure 6a and b). The nanofibrous mats supported the attachment and proliferation of MC3T3-E1 cells, an osteoblast precursor cell line, suggesting their suitability as scaffolds for bone TE. These chitosan/phosvitin-coated nanofibrous mats along with alternative mats coated with tannic acid and phosvitin exhibited antimicrobial properties, demonstrating that these constructs are versatile and could be useful for other biomedical application such as skin tissue regeneration. [193, 198]

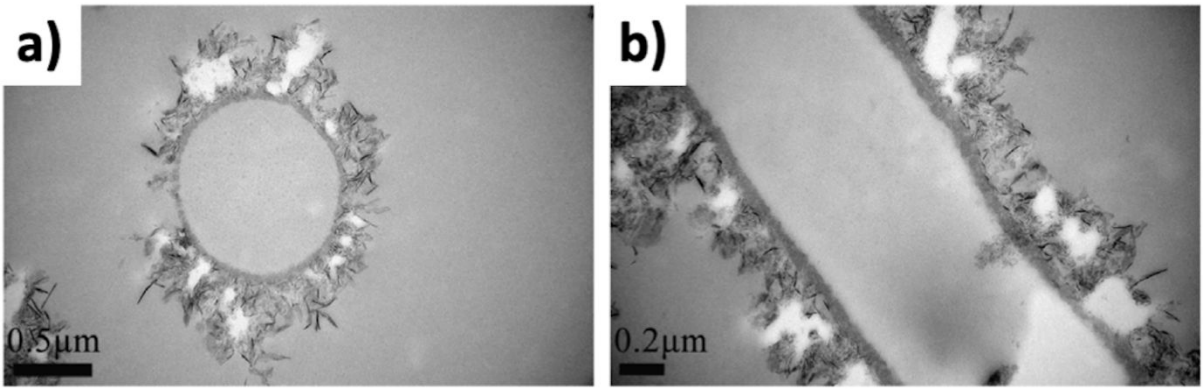


**Figure 6. Exploiting EY for TE.** Transmission electron microscopy images of a) cross section and (b) longitudinal section of a chitosan/phosvitin-coated nanofiber. Reproduced with permission from reference 197. Copyright 2017 Elsevier BV.

**Table 1.** Key strategies leveraging egg components in TE. ND: not determined.

| Egg Component | Form of Usage | Target Organ | Preparation Methods | Other Materials | Cells | Animal Models | Observations | Ref. |
|---|---|---|---|---|---|---|---|---|
| ES | CaP | Bone | 3D printing | Chitosan | MSCs | Rabbit | ES-derived CaP showed higher biological activities compared to synthetic CaP. The 3D-printed scaffolds showed promising biocompatibility *in vivo* | 66 |
| ES | HAp | Bone | Sonochemical isolation for HAp synthesis; freeze drying for scaffold preparation | Keratin and collagen | hADSCs | ND | hADSCs were successfully kept alive on the scaffolds *in vitro* and subsequently self-differentiated into osteogenic lineage without any induction agents | 64 |
| ES | HAp | Bone | Wet chemical precipitation method for HAp synthesis; blending and film forming for HAp/PCL composite scaffold preparation | PCL | Human osteosarcoma cells | ND | ES-derived HAp improved PCL scaffold swelling and degradation ratio | 96 |
| ES | HAp | Bone | Wet chemical precipitation method for HAp synthesis; blending and film forming for chitosan/HAp composite scaffolds | Chitosan | Human osteosarcoma cell line | ND | HAp improved chitosan film roughness and cell viability | 97 |
| ES | ES microparticles | Bone | Blending of materials | GelMA | MC3T3-E1 pre- | Wistar rat | ES particles significantly improved the mechanical properties of GelMA hydrogels and the differentiation and mineralization of pre- | 72 |

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| | | | | | | osteoblast cells | | osteoblasts*; in vivo* subcutaneous implantation of ESP hydrogels exhibited suitable biocompatibility | |
| | ES | HAp | Bone | Sonochemical method for HAp synthesis; blending for HAp/nano-cellulose composite scaffolds | Cellulose nanocrystals; semicrystalline cellulose nanofibrils | Human osteoblast cells | ND | HAp improved the mechanical properties of composite scaffolds and promoted osteoblast cell viability | 87 |
| | ES | βTCP | Bone | Ball milling process followed by a wet chemical precipitation method for βTCP synthesis; sponge replication method for scaffold preparation | PVA and PU | hADSCs | ND | Scaffolds showed good biocompatibility toward hADSCs | 101 |
| | ES | HAp | Bone | Wet chemical precipitation method for HAp synthesis; 3D printing for scaffold preparation | PCL | Human osteoblast cells | ND | HAp improved cell adherence and proliferation | 8 |
| | ES | βTCP | Bone | Ceramic scaffolds using sintering method | HAp | ND | ND | Increasing HAp content increased ceramic hardness | 103 |
| | ES | βTCP | Bone | Composite nanofibrous scaffolds using electrospinning technique | Carbon dots, PCL, and PVA | Human buccal fat pad-derived stem cells | ND | βTCP significantly improved cell proliferation and osteogenic differentiation | 106 |

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|---|---|---|---|---|---|---|---|---|
| ESM | ESM | Skin | Pulsed laser deposition technique over ESM films for scaffold preparation | Copper, bioactive glasses | HUVECs | Mouse | Scaffolds enhanced angiogenesis and wound healing | 199 |
| ESM | ESM | Skin | Sulfitolysis method for producing SEP; blending and electron beam irradiation for hydrogel production | PVA | ND | ND | The applied strategy displayed a suitable method for hydrogel production; scaffolds exhibited excellent absorption capacity | 200 |
| ESM | ESM | Nerve | Vacuum infiltration technique to create a double network fibrous scaffold | PCLF | PC12 | ND | ESM/PCLF double network scaffolds showed significant improvement in cell proliferation | 130 |
| ESM | ESM | Skin | Acid and alkali treatments for ESM preparation; nanocomposite film for scaffold fabrication | Silver nanoparticles (Ag-NPs) | Fibroblasts | Mouse | The fabricated scaffolds exhibited good biocompatibility, antibacterial activity, anti-inflammatory properties, and improved wound healing | 129 |
| ESM | ESM | Skin | ESM/chitosan blend film | Chitosan | ND | ND | Incorporation of ESM within the scaffold improved wettability, fluid uptake, and antibacterial activity | 115 |
| ESM | Processed ESM Powder (PEP) | Skin | Washed, milled, sieved, and γ-sterilized to produce PEP | ND | MMP-2<br>MMP-9 | Mouse | PEP significantly improved wound healing | 127 |
| ESM | SEP | Cartilage | Acid treatment for SEP preparation; blending and hydrogel preparation using | Chitosan, silk fibroin | Normal human articular | ND | These hydrogels showed proper mechanical properties and biocompatibility | 125 |

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| | | | sodium triphosphate pentabasic | | chondrocyte cells | | | |
| ESM | ESM | Skin | Acid treatment for SEP extraction; electrospinning for scaffold fabrication | Chitosan and PCL | Human dermal fibroblast cells | Mouse | The scaffolds enhanced cell adhesion and wound healing efficacy | 131 |
| ESM | SEP | Skin | Nanofibrous scaffolds using electrospinning technique | PCL, silk fibroin, and aloe vera | Human basal cells | ND | The addition of SEP improved scaffold wettability and cell viability | 138 |
| ESM | ESM | Skin | Dopamine-functionalized ESM was coated by hyaluronic acid and KR-12 peptide | Dopamine, hyaluronic acid, and KR-12 | HUVECs and human keratinocyte cells | Rat | Modified ESM enhanced the proliferation of cells *in vitro* and accelerated re-epithelialization, granulation tissue formation, and wound healing *in vivo* | 201 |
| ESM | SEP | ND | Enzymochemical method for SEP preparation; layer-by-layer film forming for scaffold production | Fe(III)-tannic acid | HeLa cells and primary hippocampal neuron cells | ND | Scaffolds showed great cytocompatibility *in vitro* | 140 |
| ESM | ESM | Bone | HAp was coated on ESM by a biomineralization method | HAp | MC3T3-E1 cells | ND | The prepared scaffolds were suitable for bone TE | 134 |

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| ESM | PEP | ND | Blending and freeze-drying to fabricate sponge scaffolds | Collagen | Human dermal fibroblasts, myofibroblast, and bovine muscle cells | ND | PEP improved the scaffolds' mechanical properties and cell adhesion | 126 |
| ESM | ESM | Vessel | ESM was coated by poly dopamine and heparin and then PU was electrospun onto its surface | PU, dopamine, heparin | HUVECs | ND | Scaffolds containing ESM exhibited higher biocompatibility | 132 |
| ESM | ESM | Vessel | Blending | Expanded polytetrafluoroethylene (ePTFE), heparin, and ethanol | HUVECs | ND | The addition of ESM enhanced the biocompatibility of ePTFE | 123 |
| ESM | SEP | Cartilage | Blending | Agarose | Rabbit Cartilage Cells | ND | SEP improved cell proliferation and increased chondrogenic gene expression | 144 |
| EW | EW | Soft tissues | Sponge-like scaffolds were prepared via carbodiimide-mediated-mediated crosslinking | 1-ethyl-3-(3-dimethylaminopropyl) carbodiimi | Human dermal fibroblasts | Nude mouse | EW improved angiogenesis and tissue regeneration *in vivo* | 151 |

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| | | | | de (EDC) and N-hydroxysuccinimide (NHS) | | | | |
| EW | Lysozyme | Bone | Coating | PEG and PCL | human osteoblast cells and MSCs | ND | The coating significantly enhanced the composite surface antibacterial activity | 176 |
| EW | EW | ND | Blending and film forming | Silk fibroin | HUVECs | ND | The addition of EW improved cell viability | 178 |
| EW | EW | Skin | EW adhesive glue was prepared and coated on PCL nanofibers | PCL | ND | Rat | PCL-coated EW glue showed promising wound healing *in vivo* | 153 |
| EW | Lysozyme | Bone | Lysozyme and glycol chitosan were methacrylated and crosslinked using riboflavin and visible light | Glycol chitosan | MSCs | Rat | The presence of lysozyme enhanced scaffold performance *in vitro* and *in vivo* | 177 |
| EW | Ovotransferrin | Bone | ND | ND | The murine osteoblastic cell line MC3T3-E1 | ND | Ovotransferrin improved cell proliferation, differentiation, and mineralization | 202 |

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|---|---|---|---|---|---|---|---|---|
| EW | Albumen | ND | Nanofibrous mat fabricated by electrospinning | PEO | ND | ND | Egg albumen was successfully formulated with PEO to fabricate fine nanofibers | 150 |
| EW | Ovalbumin | Bone | EW ovalbumin was polymerized using diethylenetriamine and injected into a printed negative mold to fabricate 3D scaffolds | HAp | ND | ND | 3D EW ovalbumin/HAp composite scaffolds were successfully produced and exhibited promising mechanical properties | 179 |
| EW/ES | EW and ES | Bone | GelMA, EW, and ES powder were blended and photocrosslinked using Irgacure 2959 and UV light | GelMA | hDPSCs | ND | The incorporation of EW and ES into GMA hydrogels improved their physical and biological properties | 180 |
| EW | EW | ND | EW was blended with gellan gum and then thermally crosslinked to form hydrogels | Gellan gum | ND | ND | The addition of gellan gum reduced the degradation of EW gels | 155 |
| EY | Phosvitin | ND | Phosvitin and chitosan were sequentially coated on cellulose acetate nanofibrous mat | Chitosan and cellulose acetate | ND | ND | The fabricated nanocomposite scaffolds possessed suitable antibacterial activity | 198 |
| EY | EY oil | Skin | EY heated to 200 °C to gain EY oil, which was then added to chitosan | Chitosan | ND | Rat | Scaffolds showed proper wound healing properties | 203 |
| EY | Phosvitin | Skin | Phosvitin and tannic acid were sequentially coated on | Tannic acid and | ND | ND | Tannic acid/phosvitin-coated cellulose acetate-coated nanofibrous mats showed good antioxidant activity | 193 |

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| | | | cellulose acetate nanofibrous mat | cellulose acetate | | | | |
| EY | EY plasma and granules | Skin | Protein gels obtained via transglutaminase crosslinking | Transglutaminase | Murine fibroblast | ND | The hydrogels showed good biocompatibility | [189] |
| EY | Phosvitin | Bone | Phosvitin and chitosan were sequentially coated on cellulose acetate nanofibrous mat | Chitosan | Mouse MC3T3-E1 pre-osteoblast cell line | ND | Phosvitin/chitosan-coated scaffolds displayed enhanced biocompatibility, cell proliferation, adhesion, and spreading as well as improved mineralization | [197] |

# 6. Conclusions and future perspectives

The avian egg, an affordable food product of high nutritional value for humans, is a remarkable source for biomaterial extraction. Each part of the egg contains bioactive elements with great potential for TE and regenerative medicine (Table 1). Regarding the use of avian egg components (particularly those from chickens) as a source of biomaterials, several challenges remain. As with other naturally derived biomaterials, batch-to-batch variations exist and arise through multiple causes such as the type of food eaten by the hens. Consequently, it is necessary to establish protocols and minimize variables that lead to such disparities, ultimately ensuring reproducibility and reliability. Moreover, suitable sterilization techniques should be investigated for use with egg components in TE. The effects of sterilization techniques on egg components for food industries have been well studied, but only limited data are available regarding the effect of these techniques on the material properties and the application of egg components for TE. Additionally, specific limitations exist for the current egg-derived scaffolds. For instance, the insolubility of ESM restricts its widespread application. Although various methods are available to produce soluble ESM, a mild reaction procedure without the use of toxic chemicals and/or high temperatures is yet to be discovered. With respect to EY, more work is needed to fully investigate its potential application in TE and beyond. Importantly, many scaffolds containing egg components have yet to be tested in relevant and large animal models, a critical step for translational research and progression to the first stage of clinical testing in humans.

Although egg-derived biomaterials are still under-exploited, the latest advancements in the field of TE and other biomedical fields within the past few years have opened new opportunities for their widespread use. Bioinspired egg-derived scaffolds may have important applications in the development of organ-on-a-chip systems to further integrate 3D cell culture,

microfluidic technology, and TE for disease modelling, drug discovery, and personalized medicine. Lastly, combining these naturally derived biomaterials with state-of-the-art technologies such as 3D bioprinting may lead to the fabrication of sophisticated tissue-engineered constructs, ultimately leading to more functional and viable tissues and organs.

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## Conflict of Interest

The authors declare no conflict of interest.

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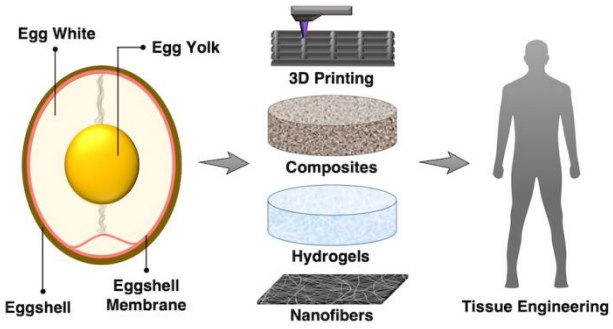
# Table of content graphic



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